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Construction and synthesis of tricyclic matrinic derivatives against influenza A virus by privileged structure strategy

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A series of new matrinic derivatives with an 11-adamantyl group were designed, synthesized and evaluated for their anti-influenza A H3N2 activities, based on the privileged structure strategy. SAR analysis indicated that introduction of an 11-adamantyl by ester linker might be helpful for the activity. Among them, compound **7b** exhibited promising anti-H3N2 activities with IC_{50} value of 5.14 μM, slightly better than that of amantadine. Its activity was further confirmed at the protein level. In primary mechanism, compound **7b** could inhibit virus replication cycle at early stage by targeting M2 protein, consistent with that of amantadine. This study represents a successful application of combined strategy of privileged amantadine segment for further structural optimization and development of a new class of anti-influenza agents.

1. Introduction

Influenza A virus, a highly pathogenic virus to human beings, is most susceptible to mutation and thus causes rapid and severe global pandemics, resulting in millions of fatalities worldwide (Zambon et al. 2014; Zhang et al. 2018; Coleman et al. 2018). According to the World Health Organization, there are approximately 3 to 5 million cases of severe influenza infections and up to 500,000 deaths each year (Ali et al. 2018). Influenza A H3N2 and H1N1 all belong to highly pathogenic influenza A viruses, and H1N1 is even worse (Phipps et al. 2017). However, the incidence of influenza A H3N2 has been exceeding that of H1N1 since 2010, becoming the most prevalent virus across the world (Zhu et al. 2015). Though influenza vaccination is one strategy for controlling influenza infections, live attenuated and inactivated influenza vaccines often show a decrease or even loss of productivity and efficiency owing to antigenic drift and shift (Kirkdale et al. 2017; Liu et al. 2018). Several anti-influenza virus drugs (Gu et al. 2013; Leneva et al.2016; Amarelle et al.2017; Cohen et al. 2018), such as NA inhibitors and M2 proton channel blockers, have been using in clinic, however an increasing number of drug resistant variants of influenza viruses appeared, especially oseltamivir resistant strains (Moscona 2009). Therefore, it is urgently needed to develop innovative inhibitors with new structures or novel modes of action to combat the influenza virus.

In our previous study, a specific tricyclic matrinic compound **1** (Fig. 1), derived from the natural product matrine (**MT**), was first identified to have a moderate activity against influenza A virus with IC_{50} of 16.2 μM, with unknown mechanism of action (Tang et al. 2018). Its unique chemical scaffold and specific activity against influenza encouraged us to further explore the structure-activity relationship (SAR) of its kind, in an effort to get antivirus candidates against influenza. As we all know, amantadine and rimantadine, belonging to M2 proton channel blockers, have been widely used in clinical practice for decades. The results indicated that the amantadine scaffold with rigid structure could be used as a privileged segment for linking with a tricyclic matrinic core to generate hybrid compounds against influenza viruses, as depicted in Fig. 1. In addition, according to our previous SAR results, the methylene (CH_2) linker on 12N-atom was replaced by a sulfonyl (SO_2) group as a druglike feature (Cheng et al. 2017; Tang et al. 2018), by which a series of novel 12*N*-benzenesulfonyl matrinic derivatives were constructed and synthesized. Here we describe the synthesis of novel sixteen 12*N*-subsitituted benzenesulfonyl matrinic derivatives, anti-influenza activity and SAR analysis, as well a primary mechanism of action suggestion for the key compound.

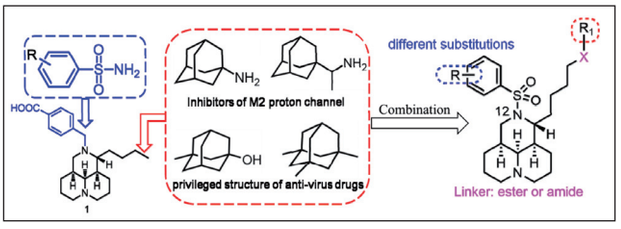
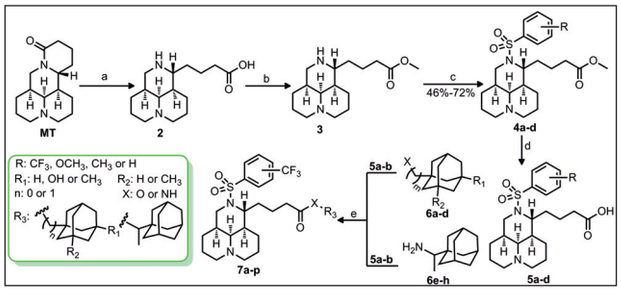


Fig. 1: Chemical structure of **1** and structure modification strategy

2. Investigations, results and discussion

2.1. Chemistry

As described in the Scheme, all the title compounds were synthesized from commercially available matrine (**MT**) with purity over 98 %, which was purchased from the Yanchi Dushun Biological and Chemical Co. Ltd (Shanxi, China). The matrinic ester **3** was obtained in a two-step procedure, including hydrolysis and esterification from **MT** with a yield of 85 %. The key intermediates **5a–d** were acquired by 12N-sulfonylation and ester hydrolysis from **3** in yields of 65–70 % (Li et al. 2017). The title compounds **7a–p** were synthesized by carboxyl esterification or amidation of the intermediates **5a–d** with compounds **6a–h**. A the compounds were purified with flash column chromatography on silica gel using dichloromethane/methanol as eluent.



Scheme: (a) 5 N NaOH, reflux, 9 h; 12 N HCl, pH < 4 ; (b) 2 N MeOH/HCl, reflux, 2 h; (c) substituted benzensulfonyl chloride, TEA, CH_2Cl_2, r.t., 6–8 h; (d) 5 N NaOH, reflux, 1-2 h, 2 N HCl, pH = 4–5; (e) $SOCl_2$, CH_2Cl_2, TEA, r.t., 1 h.

2.2. Pharmacology

2.2.1. SAR analysis for anti-H3N2 activity in vitro

For all the target compounds, activities against infuenza A/hanfang/359/95 (H3N2) were measured by viral cytopathogenic effect (CPE) assay in Madin-Darby camine kidney (MDCK) cells with amantadine hydrochloride (**AH**) as control drug. The activity against H3N2 of each compound was evaluated by combination of its IC_{50} and selectivity index (SI = TC_{50}/IC_{50} ratio). The structures and the activities of all title compounds against H3N2 were shown in the Table.

First, an adamantyl group was linked to C11–side chain by an ester bond and different benzenesulfonyl groups were respectively introduced on 12N atom, by which four 12*N*-benzenesulfonyl matrinane derivatives (**7a–d**) were generated and evaluated for activity against H3N2. All of them displayed activities comparable to or higher than the lead **1**, especially compound **7b** with *m*-trifluoromethyl on the benzene ring was more active than compound **1** and **AH**, with an IC_{50} value of 5.14 μM.

Moreover, a trifluoromethylbenzenesulfonyl group at the 12N-position was retained, and different substituted adamantyl was linked to C11–side chain terminus by an ester bond, by which compounds **7e–i** were constructed. As depicted in the Table 1, compounds **7f–h** with 3'-hydroxy adamantyl or adamantylethyl afforded comparable activity to compound **1**, while compounds **7e** and **7h** with 3',5'-dimethyl adamantyl showed a complete loss in activity. Furthermore, an adamantyl group on C11–side chain was maintained, the linker of an ester bond was converted to an amido bond on C11–side chain, by which corresponding compounds **7j-p** were produced. All the compounds had lost their activities against influenza A H3N2 virus completely. Therefore, the results indicated that linking adamantyl to the C11–side chain terminus by an ester bond might be beneficial to retain or enhance the potency against influenza A H3N2.

2.2.2. Anti–H3N2 activity of compound *7b* at protein level

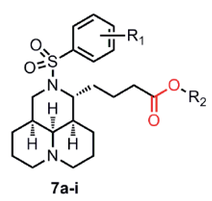
In order to confirm the anti–H3N2 effect of this kind of compounds, the compound **7b** with the highest anti-H3N2 was selected to confirm its effect against influenza A H3N2 virus at protein level by Western Blot assay, taking **AH** as a positive reference. As indicated in Fig. 2, compound **7b** could significantly reduce H3N2 virus non–structural protein 1 (NS1) level at the concentration of 40 μM. The results indicated that **7b** was a potential inhibitor of influenza A virus H3N2 at protein level.

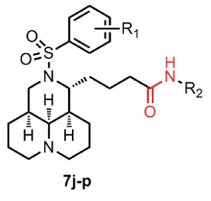
2.2.3. The primary mechanism of action of compound *7b*

To investigate the primary mechanism of this kind of compounds, the inhibition of 7**b** for matrix protein 2 was tested by Western Blot. As showed in Fig. 2, compound **7b** could significantly reduce the matrix protein 2 level at a concentration of 40 μM, similar to that of **AH**.

Furthermore, a time-of-addition experiment of **7b** was carried out. The therapeutic efficacy of **7b** at different infection time points (before, at and after infection) was measured and the results are shown in Fig. 3. Compound **7b** was effective at –2 h, 0 h and 2 h of H3N2 infection, and no significant changes were observed in the later periods, indicating that compound **7b** might inhibit virus replication cycle at early stage, consistent with that of **AH**.

Table 1 Anti-H3N2 activities of all title compounds





Comp.	R_1	R_2	TC_{50} (μM) [a]	A/hanfang/359/95	
				IC_{50} (μM) [b]	SI [c]
1			> 540.5	16.2	> 33.4
7a	*p*–CF_3		98.4±9.68	21.1±3.91	4.67
7b	*m*–CF_3		47.8±9.29	5.14±0.4	9.31
7c	*p*-CH_3		53.1±0	16.2±0	3.28
7d	H		44.7±7.65	16.3±1.83	2.75
7e	*p*–CF_3		2.84±0.20	–[d]	–
7f	*p*–CF_3		14.9±1.65	9.53±0.47	1.57
7g	*p*–CF_3		71.4±12.5	16.9±1.55	4.22
7h	*m*–CF_3		57.1±0.00	11.1±1.84	5.16
7i	*m*–CF_3		70.2±6.82	–	–
7j	*p*–CF_3		109.1±15.86	–	–
7k	*p*–CF_3		3.58±0.00	–	–
7l	*p*–CF_3		1.28±0.21	–	–
7m	*p*–CF_3		18.1±0	–	–
7n	*m*–CF_3		18.8±1.42	–	–
7o	*p*–CF_3		4.20±0.19	–	–
7p	*m*–CF_3		2.91±0.40	–	–
AH			>1069	17.4±2.62	>61.54

[a] 50% cytotoxic concentration (μM); [b] 50% virus–inhibitory concentration (μM); [c] Selectivity Index values equaled to TC_{50}/IC_{50}; [d] no activity.

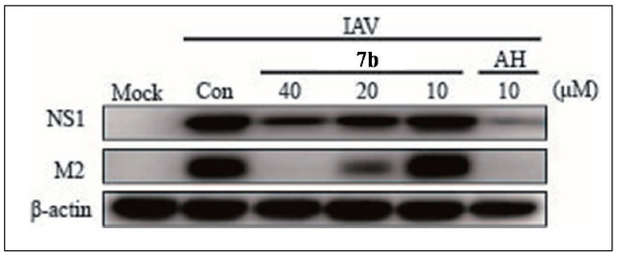


Fig. 2: Antiviral effect of **7b** at protein level

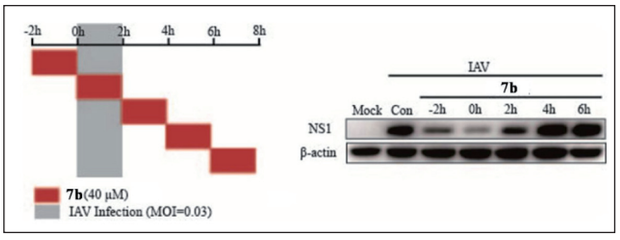


Fig. 3: In time-of-addition studies of **7b**

2.3. Conclusion

A series of new matrinic derivatives with an 11-adamantyl group were synthesized and evaluated for their anti-H3N2 activities. SAR indicated that linking adamantyl to the C11-side chain terminus by an ester bond might be beneficial for the activity. Among them, compound **7b** exhibited promising anti-H3N2 activities with IC_{50} value of 5.14 μM, and SI value of 9.31, better than that of lead **1** and control **AH**. Its effect against influenza A H3N2 was further confirmed at protein level. In primary mechanism, compound **7b** could inhibit virus replication cycle at early stage by targeting M2 protein, consistent with that of **AH**. This study provides a successful application for combined strategy of privileged adamantyl segment for further structural optimization and development of tricyclic matrinic derivatives into a new class of anti-influenza agents.

3. Experimental

3.1. Chemistry

Unless otherwise noted, all chemical reagents and anhydrous solvents were obtained from commercial sources and used without further purification. Melting points (m.p.) were obtained with a CXM–300 melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer (Varian, San Francisco, USA) (600 MHz and 500 MHz for ^{1}H NMR; 151 MHz and 126 MHz for ^{13}C NMR) in DMSO–d_6 with Me_4Si as the internal standard. ESI high–resolution mass spectras (HRMS) were recorded on an AutospecUltima–TOF spectrometer (Micromass UK Ltd., Manchester, UK). Flash column chromatography was performed on Combiflash Rf 200 (Teledyne, NE, USA), particle size 0.038 mm.

3.1.1. Synthesis of compounds 4a–d

MT (10.0 g, 40 mmol) was added to 5 N NaOH solution (50 mL), and the resulting mixture was refluxed for 9 h, cooled overnight to room temperature and many solids have been precipitated. Then transferred the solid into concentrated HCl (10 N) and kept pH of the solution less than 4. The solvent was removed in vacuo and the residue was dissolved with methanol (50 mL). Filtered the suspension, and the filtrate was concentrated. The residue was dissolved in 2 mol/L HCl/MeOH (50 mL) and the mixture was stirred at room temperature for 8 h. The solvent was removed under reduced pressure to give a crude product, which was purified by recrystallization from ethanol to achieve intermediate **3** (13.1 g).

To a suspension of compound **3** (10.0 g, 28 mmol) in dichloromethane (50 mL), triethylamine (8.68 g, 86 mmol) and *p*-trifluoromethylbenzenesulfonyl chloride (34 mmol) were added, the reaction mixture was stirred at room temperature for 6 h until TLC analysis showed completion of the reaction. The reaction mixture was then washed by saturated aqueous ammonium chloride (50 mL × 2) and saturated aqueous sodium chloride (50 mL) subsequently, dried over anh. sodium sulfate and concentrated under reduced pressure. The residue was purified by recrystallization from methanol to give **4a**. The compounds **4b-d** can be synthesized from **3** with corresponding benzenesulfonyl chloride as the same manner.

3.1.2. Synthesis of compounds 5a–d

Compound **4a** (10 mmol) were refluxed in 5 N NaOH solution (30 mL) for 4 h, cooled and acidified with HCl (2 N) to pH 4–5. The solvent was removed by condensation, and the residue was suspended in methanol, the precipitate was filtered off. Filtrated cake was dissolved in dichloromethane and dried over anhydrous sodium sulfate. The solution was concentrated under reduced pressure, and the crude product **5a** was used directly without further isolation. The compounds **5b-d** can be obtained in the same manner as **5a**.

3.1.3. Synthesis of compounds 7a–p

Compounds **5a**–**b** (1.1 mmol) were dissolved in thionyl chloride (5 mL), and the mixture was stirred for 1 h. Then the solvent was removed by condensation, the residue was dissolved in dichloromethane (10 mL) which was added in to a solution of **6a**–**h** (1.2 mmol) and triethylamine (1.5 mmol) in dichloromethane (30 ml), and the solution was stirred at room temperature until the TLC showed completion of the reaction. The solution was washed by saturated aqueous ammonium chloride (50 mL x 2), saturated aqueous sodium chloride (50 mL), dried over anh. sodium sulfate, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel with dichloromethane/methanol as the eluent and treated with 2 N hydrochloride/ether (3 mL) to give the desired products.

1'-*Adamantanemethyl*-12*N*-*p*-*trifluoromethlybenzenesulfonyl matrinate hydrochloride* (**7a**): Yield: 63 %; m.p.: 225 °C (dec.); ^{1}H NMR (600 MHz) δ 10.64 (s, 1H), 8.06 (d, *J* = 8.2 Hz, 2H), 7.95 (d, *J* = 8.2 Hz, 2H), 3.84–3.76 (m, 2H), 3.59–3.56 (m, 1H), 3.54 (d, *J* = 3.0 Hz, 2H), 3.20 (d, *J* = 11.9 Hz, 2H), 2.93–2.85 (m, 2H), 2.29–2.19 (m, 3H), 2.10–2.04 (m, 1H), 1.94–1.83 (m, 5H), 1.75–1.52 (m, 15H), 1.43 (d, *J* = 2.8 Hz, 6H), 1.39–1.31 (m, 1H), 1.18–1.10 (m, 1H); ^{13}C NMR (151 MHz) δ 172.4, 145.2, 132.4, 127.6 (2), 126.6 (2), 123.4, 72.8, 62.9, 57.5, 54.6, 54.5, 48.6, 38.6 (3), 38.1, 36.4 (3), 34.7, 33.0, 32.7, 27.4 (3), 27.2, 24.8, 24.3, 20.9, 18.3, 18.1; ESI-HRMS: *m/z* Calcd for $C_{33}H_{46}O_4N_2F_3S$ [M-HCl+H]$^+$, 623.3125; Found, 623.3121.

1'-*Adamantanemethyl*-12*N*-m-*trifluoromethlybenzenesulfony matrinate hydrochloride* (**7b**): Yield: 55 %; m.p.: 109–110 °C; ^{1}H NMR (600 MHz) δ 10.85 (s, 1H), 8.18 (d, *J* = 7.9 Hz, 1H), 8.06–8.01 (m, 2H), 7.85 (t, *J* = 7.9 Hz, 1H), 4.27–4.21 (m, 1H), 3.92–3.79 (m, 2H), 3.60 (d, *J* = 10.0 Hz, 1H), 3.53 (s, 2H), 3.19 (d, *J* = 11.7 Hz, 2H), 2.94–2.85 (m, 2H), 2.33–2.15 (m, 3H), 2.06–1.99 (m, 1H), 1.94–1.86 (m, 5H), 1.79–1.53 (m, 14H), 1.42 (d, *J* = 2.8 Hz, 6H), 1.31–1.25 (m, 1H), 1.10–1.03 (m, 1H); ^{13}C NMR (151 MHz) δ 172.3, 143.0, 131.1, 130.7, 129.9, 129.4 122.9, 122.3, 72.8, 63.0, 57.9, 54.6, 54.5, 48.8, 38.6 (3), 38.2, 36.4 (3), 35.0, 33.0, 32.7, 27.4 (3), 26.9, 24.8, 24.3, 21.2, 18.3, 18.0; ESI-HRMS: *m/z* Calcd for $C_{33}H_{46}O_4N_2F_3S$ [M-HCl+H]$^+$, 623.3125; Found, 623.3103.

1'-*Adamantanemethyl*-12*N*-*p*-*methlybenzenesulfonyl matrinate hydrochloride* (**7c**): Yield: 46 %; m.p.: 174–175 °C; ^{1}H NMR (500 MHz) δ 10.14 (s, 1H), 7.72 (d, *J* = 8.1 Hz, 2H), 7.39 (d, *J* = 8.1 Hz, 2H), 3.92 (dt, *J* = 11.6, 6.3 Hz, 1H), 3.73–3.61 (m, 2H), 3.57–3.50 (m, 3H), 3.21 (d, *J* = 11.9 Hz, 2H), 2.94–2.84 (m, 2H), 2.38 (s, 3H), 2.28–2.15 (m, 3H), 2.12–2.02 (m, 1H), 1.96–1.91 (m, 3H), 1.89–1.78 (m, 2H), 1.71–1.56 (m, 14H), 1.49–1.34 (m, 7H), 1.25–1.15 (m, 1H); ^{13}C NMR (151 MHz) δ 172.2, 143.1, 138.9, 129.7 (2), 126.7 (2), 73.9, 65.8, 58.3, 56.5, 56.4, 55.7, 49.5, 39.3 (3), 38.7, 37.0 (3), 35.4, 33.8, 33.2, 28.1 (3), 27.9, 25.8, 25.3, 21.7, 18.9, 18.7; ESI-HRMS: *m/z* Calcd for $C_{33}H_{49}O_4N_2S$ [M-HCl+H]$^+$, 569.3408; Found, 569.3407.

1'-*Adamantanemethyl*-12*N*-*benzenesulfonyl matrinate hydrochloride* (**7d**): Yield: 52 %; m.p.: 166–167 °C; ^{1}H NMR (500 MHz) δ 10.52 (d, *J* = 11.7 Hz, 1H), 7.86–7.81 (m, 2H), 7.68–7.62 (m, 1H), 7.58 (dd, *J* = 8.3, 6.8 Hz, 2H), 4.11–4.03 (m, 1H), 3.81–3.69 (m, 2H), 3.60–3.53 (m, 3H), 3.19 (d, *J* = 11.8 Hz, 2H), 2.94–2.84 (m, 2H), 2.29–2.14 (m, 3H), 2.10–2.00 (m, 1H), 1.96–1.91 (m, 3H), 1.91–1.82 (m, 2H), 1.77–1.53 (m, 14H), 1.44 (d, *J* = 3.0 Hz, 6H), 1.37–1.27 (m, 1H), 1.20–1.10 (m, 1H); ^{13}C NMR (126 MHz) δ 172.6, 141.6, 132.8, 129.4 (2), 126.6 (2), 72.8, 63.0, 57.4, 54.6, 54.5, 48.4, 38.7 (3), 37.8, 36.4 (3), 34.5, 33.1, 32.8, 27.4 (3), 27.1, 24.9, 24.4, 21.0, 18.3, 18.1; ESI-HRMS: *m/z* Calcd for $C_{32}H_{47}O_4N_2S$ [M-HCl+H]$^+$, 555.3251; Found, 555.3241.

1'-*(3,5-Dimethyladamantyl)*-12*N*-*p*-*trifluoromethlybenzenesulfonyl matrinate hydrochloride* (**7e**): Yield: 67 %; m.p.: 151–152 °C ; ^{1}H NMR (500 MHz) δ 10.66 (d, *J* = 12.2 Hz, 1H), 8.06 (d, *J* = 8.2 Hz, 2H), 7.95 (d, *J* = 8.2 Hz, 2H), 4.13 (dt, *J* = 11.8, 5.8 Hz, 1H), 3.87–3.74 (m, 2H), 3.61–3.55 (m, 3H), 3.19 (d, *J* = 11.8 Hz, 2H), 2.94–2.83 (m, 2H), 2.29–2.25 (m, 1H), 2.25–2.17 (m, 2H), 2.11–2.03 (m, 1H), 2.03–1.97 (m, 1H), 1.91–1.82 (m, 2H), 1.72–1.51 (m, 8H), 1.39–1.31 (m, 1H), 1.29–1.24 (m, 6H), 1.17–1.01 (m, 7H), 0.78 (s, 6H); ^{13}C NMR (126 MHz) δ 172.5, 145.2, 132.5, 127.7 (2), 126.6 (2), 123.5, 72.3, 62.9, 57.6, 54.6, 54.5, 50.6, 48.6, 44.9 (2), 42.7 (2), 38.1, 37.3, 34.7, 34.6, 32.9, 30.5 (2), 30.4 (2), 28.6, 27.2, 24.8, 24.3, 21.0, 18.3, 18.1; ESI-HRMS: *m/z* Calcd for $C_{35}H_{50}O_4N_2F_3S$ [M-HCl+H]$^+$, 651.3437; Found, 651.3448.

1'-*(3-Hydroxyadamantanemethyl)*-12*N*-*p*-*trifluoromethlybenzenesulfonyl matrinate hydrochloride* (**7f**): Yield: 67 %; m.p.: 245 °C (dec.); ^{1}H NMR (500 MHz) δ 10.51 (d, *J* = 11.8 Hz, 1H), 8.06 (d, *J* = 8.3 Hz, 2H), 7.97 (d, *J* = 8.3 Hz, 2H), 4.09 (dt, *J* = 11.6, 5.8 Hz, 1H), 3.79 (d, *J* = 8.6 Hz, 2H), 3.61–3.55 (m, 3H), 3.20 (d, *J* = 11.9 Hz, 2H), 2.93–2.85 (m, 2H), 2.30–2.17 (m, 3H), 2.12–2.02 (m, 3H), 1.92–1.80 (m, 2H), 1.70–1.44 (m, 14H), 1.39–1.25 (m, 8H), 1.19–1.07 (m, 1H); ^{13}C NMR (126 MHz) δ 172.5, 145.1, 132.5, 127.7 (2), 126.7 (2), 123.4, 72.1, 66.4, 62.9, 57.5, 54.6, 54.5, 48.5, 46.7, 44.6, 38.0 (2), 37.7, 36.3 (2), 35.3, 34.6, 33.0, 29.6 (2), 27.2, 24.8, 24.3, 20.9, 18.3, 18.1; ESI-HRMS: *m/z* Calcd for $C_{33}H_{46}O_5N_2F_3S$ [M-HCl+H]$^+$, 639.3074; Found, 639.3078.

1'-*Adamantanethyl*-12*N*-*p*-*trifluoromethlybenzenesulfonyl matrinate hydrochloride* (**7g**): Yield: 69 %; m.p.: 230–231 °C; ^{1}H NMR (600 MHz) δ 10.78 (s, 1H), 8.06 (d, *J* = 8.2 Hz, 2H), 7.95 (d, *J* = 8.2 Hz, 2H), 4.20–4.15 (m, 1H), 3.96 (t, *J* = 7.5 Hz, 2H), 3.86 (t, *J* = 13.2 Hz, 1H), 3.82–3.77 (m, 1H), 3.61–3.56 (m, 1H), 3.19 (d, *J* = 11.8 Hz, 2H), 2.93–2.86 (m, 2H), 2.29–2.23 (m, 2H), 2.21–2.15 (m, 1H), 2.01–1.94 (m, 1H), 1.92–1.83 (m, 5H), 1.72–1.52 (m, 14H), 1.48–1.43 (m, 6H), 1.33–1.26 (m, 3H), 1.10–1.04 (m, 1H); ^{13}C NMR (151 MHz) δ 172.3, 145.3, 132.4, 127.6 (2), 126.5 (2), 123.4, 62.9, 59.8, 57.7, 54.6, 54.5, 48.7, 41.9, 41.8 (3), 38.1, 36.4 (3), 34.9, 33.1, 31.2, 27.9 (3), 27.0, 24.8, 24.2, 21.0, 18.3, 18.0; ESI-HRMS: *m/z* Calcd for $C_{34}H_{48}O_4N_2F_3S$ [M-HCl+H]$^+$, 637.3181; Found, 637.3163.

1'-*(3-Hydroxyadamantanemethyl)*-12*N-m*-trifluoromethlybenzenesulfonyl *matrinate hydrochloride* (**7h**): Yield: 59 %; m.p.: 111–112 °C ; ^{1}H NMR (500 MHz) δ 10.59 (d, *J* = 9.9 Hz, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 8.08–8.03 (m, 2H), 7.86 (t, *J* = 8.0 Hz, 1H), 4.20–4.11 (m, 1H), 3.85–3.79 (m, 2H), 3.58 (s, 3H), 3.23–3.16 (m, 2H), 2.94–2.84 (m, 2H), 2.33–2.23 (m, 2H), 2.23–2.14 (m, 1H), 2.11–2.05 (m, 2H), 2.05–1.96 (m, 1H), 1.92–1.81 (m, 2H), 1.76–1.41 (m, 15H), 1.35–1.25 (m, 7H), 1.10–1.02 (m, 1H); ^{13}C NMR (126 MHz) δ 172.4, 142.8, 131.2, 130.8, 129.9, 129.7, 123.4, 123.0, 72.1, 66.4, 63.0, 57.8, 54.6, 54.5, 48.6, 46.7, 44.6, 38.1 (2), 37.6, 36.3 (2), 35.3, 34.8, 32.9, 29.6, 27.0 (2), 24.8, 24.4, 21.2, 18.3, 18.1; ESI-HRMS: *m/z* Calcd for $C_{33}H_{46}O_5N_2F_3S$ [M-HCl+H]$^+$, 639.3074; Found, 639.3074.

1'-*(3,5-Dimethyladamantyl)*-12*N-m*-trifluoromethlybenzenesulfonyl *matrinate hydrochloride* (**7i**): Yield: 73 %; m.p.: 184–185 °C ; ^{1}H NMR (500 MHz) δ 10.77 (d, *J* = 9.5 Hz, 1H), 8.18 (d, *J* = 8.2 Hz, 1H), 8.06–8.02 (m, 2H), 7.86 (t, *J* = 8.2 Hz, 1H), 4.25–4.16 (m, 1H), 3.90–3.78 (m, 2H), 3.57 (s, 3H), 3.19 (d, *J* = 11.7 Hz, 2H), 2.95–2.83 (m, 2H), 2.32–2.23 (m, 2H), 2.21–2.13 (m, 1H), 2.05–1.96 (m, 2H), 1.92–1.83 (m, 2H), 1.74–1.51 (m, 8H), 1.31–1.23 (m, 7H), 1.14–1.01 (m, 7H), 0.78 (s, 6H); ^{13}C NMR (126 MHz) δ 172.4, 142.9, 131.2, 130.8, 129.9, 129.6, 123.4, 122.9, 72.3, 63.0, 57.9, 54.6, 54.5, 50.6, 48.7, 44.9 (2), 42.7 (2), 38.1, 37.3, 34.9, 34.6, 32.9, 30.5 (2), 30.4 (2), 28.6, 27.0, 24.8, 24.4, 21.3, 18.3, 18.1; ESI-HRMS: *m/z* Calcd for $C_{35}H_{50}O_4N_2F_3S$ [M-HCl+H]$^+$, 651.3438; Found, 651.3437.

N'-Adamantanemethyl-12N-p-trifluoromethlybenzenesulfonyl *matrinicamide hydrochloride* (**7j**): Yield: 55 %; m.p.: 186–187 °C ; ^{1}H NMR (500 MHz) δ 10.42 (d, *J* = 9.8 Hz, 1H), 8.06 (d, *J* = 8.3 Hz, 2H), 7.97 (d, *J* = 8.3 Hz, 2H), 7.64 (t, *J* = 6.2 Hz, 1H), 4.12–4.04 (m, 1H), 3.81–3.70 (m, 3H), 3.58 (dt, *J* = 10.5, 3.4 Hz, 1H), 3.24–3.18 (m, 2H), 2.95–2.84 (m, 2H), 2.76 (dd, *J* = 13.2, 6.5 Hz, 1H), 2.62 (dd, *J* = 13.2, 5.9 Hz, 1H), 2.31–2.20 (m, 2H), 2.08–1.94 (m, 2H), 1.91–1.82 (m, 5H), 1.73–1.52 (m, 13H), 1.40–1.30 (m, 7H), 1.28–1.20 (m, 1H); ^{13}C NMR (126 MHz) δ 171.8, 145.1, 132.4, 127.6 (2), 126.6 (2), 123.5, 63.0, 57.4, 54.6, 54.5, 50.0, 48.6, 39.8, 38.4, 36.6 (3), 34.5, 34.4, 33.5 (3), 27.7 (3), 27.4, 24.8, 24.3, 21.6, 18.3, 18.1; ESI-HRMS: *m/z* Calcd for $C_{33}H_{47}O_3N_3F_3S$ [M-HCl+H]$^+$, 622.3285; Found, 622.3278.

N'-Adamantyl-12N-p-trifluoromethlybenzenesulfonyl *matriniccarboxamide hydrochloride* (**7k**): Yield: 56 %; m.p.: 177–178 °C ; ^{1}H NMR (600 MHz) δ 10.56 (q, *J* = 9.9 Hz, 1H), 8.05 (d, *J* = 8.3 Hz, 2H), 7.97 (d, *J* = 8.3 Hz, 2H), 7.28 (s, 1H), 4.18–4.13 (m, 1H), 3.84–3.77 (m, 3H), 3.73 (dd, *J* = 14.0, 4.9 Hz, 1H), 3.59 (dt, *J* = 10.5, 3.4 Hz, 1H), 3.22 (d, *J* = 11.9 Hz, 2H), 2.95–2.86 (m, 2H), 2.33–2.23 (m, 2H), 2.03–1.95 (m, 4H), 1.91–1.83 (m, 9H), 1.80–1.74 (m, 1H), 1.71–1.66 (m, 2H), 1.61–1.55 (m, 9H), 1.26–1.20 (m, 2H); ^{13}C NMR (151 MHz) δ 171.0, 145.3, 132.3, 127.5 (2), 126.6 (2), 123.5, 63.1, 57.6, 54.6, 54.5, 50.5, 48.8, 40.9(3), 38.5, 36.1(3), 34.9, 34.6, 28.8(3), 27.0, 24.7, 24.3, 21.7, 18.3, 18.2; ESI-HRMS: *m/z* Calcd for $C_{32}H_{45}O_3N_3F_3S$ [M-HCl+H]$^+$, 608.3128; Found, 608.3122.

N'-(3,5-Dimethyladamantyl)-12N-p-trifluoromethlybenzenesulfonyl *matriniccarboxamide hydrochloride* (**7l**): Yield: 68 %; m.p.: 148–149 °C ; ^{1}H NMR (600 MHz) δ 10.55 (d, *J* = 9.7 Hz, 1H), 8.05 (d, *J* = 8.2 Hz, 2H), 7.97 (d, *J* = 8.2 Hz, 2H), 7.28 (s, 1H), 4.17–4.11 (m, 1H), 3.80 (t, *J* = 13.1 Hz, 1H), 3.74 (dd, *J* = 13.9, 5.0 Hz, 1H), 3.61–3.57 (m, 1H), 3.24–3.19 (m, 2H), 2.94–2.86 (m, 2H), 2.33–2.27 (m, 1H), 2.27–2.22 (m, 1H), 2.05–2.00 (m, 1H), 2.00–1.93 (m, 1H), 1.91–1.82 (m, 3H), 1.80–1.72 (m, 1H), 1.70–1.65 (m, 4H), 1.61–1.48 (m, 8H), 1.29–1.16 (m, 7H), 1.06 (s, 2H), 0.78 (s, 6H).; ^{13}C NMR (151 MHz) δ 171.1, 145.2, 132.4, 127.5 (2), 126.6 (2), 123.4, 63.1, 57.6, 54.6, 54.5, 52.1, 50.3, 48.8, 46.9(2), 42.3(2), 39.4, 38.4, 35.1, 34.6, 31.8 (2), 30.1 (2), 29.5, 27.0, 24.7, 24.3, 21.7, 18.3, 18.2; ESI-HRMS: *m/z* Calcd for $C_{34}H_{49}O_3N_3F_3S$ [M-HCl+H]$^+$, 636.3441; Found, 636.3438.

N'-(3-Hydroxyadamantyl)-12N-p-trifluoromethlybenzenesulfonyl *matriniccarboxamide hydrochloride* (**7m**): Yield: 68 %; m.p.: 166–167 °C ; ^{1}H NMR (600 MHz) δ 10.61 (d, *J* = 9.8 Hz, 1H), 8.05 (d, *J* = 8.3 Hz, 2H), 7.97 (d, *J* = 8.3 Hz, 2H), 7.37 (s, 1H), 4.20–4.14 (m, 1H), 3.81 (t, *J* = 13.2 Hz, 1H), 3.73 (dd, *J* = 14.0, 4.8 Hz, 1H), 3.61 (d, *J* = 3.5 Hz, 1H), 3.21 (d, *J* = 11.7 Hz, 2H), 2.95–2.86 (m, 2H), 2.33–2.22 (m, 2H), 2.09–2.05 (m, 2H), 2.05–1.99 (m, 1H), 1.92–1.53 (m, 19H), 1.50–1.47 (m, 3H), 1.45–1.36 (m, 2H), 1.27–1.19 (m, 2H); ^{13}C NMR (151 MHz) δ 171.1, 145.3, 132.4, 127.5 (2), 126.6 (2), 123.4, 67.2, 63.1, 57.6, 54.6, 54.5, 53.2, 49.0, 48.9, 44.2 (2), 39.8 (2), 38.5, 34.9, 34.8, 34.6, 30.0 (2), 26.9, 24.7, 24.3, 21.6, 18.3, 18.2; ESI-HRMS: *m/z* Calcd for $C_{32}H_{45}O_4N_3F_3S$ [M-HCl+H]$^+$, 624.3077; Found, 624.3074.

N'-Adamantanemethyl-12N-m-trifluoromethlybenzenesulfonyl *matrinic amide* (**7n**): Yield: 50 %; m.p.: 65–66 °C; ^{1}H NMR (500 MHz) δ 8.15 (d, *J* = 8.0 Hz, 1H), 8.06–8.01 (m, 2H), 7.83 (t, *J* = 8.0 Hz, 1H), 7.64 (t, *J* = 6.3 Hz, 1H), 3.52 (q, *J* = 6.5 Hz, 1H), 3.39–3.31 (m, 2H), 3.14–3.05 (m, 1H), 2.74 (d, *J* = 6.3 Hz, 2H), 2.35–2.29 (m, 1H), 2.25–2.20 (m, 1H), 2.07 (t, *J* = 7.1 Hz, 2H), 1.92–1.87 (m, 3H), 1.82–1.80 (m, 1H), 1.67–1.46 (m, 15H), 1.40 (d, *J* = 3.0 Hz, 6H), 1.32–1.24 (m, 4H), 1.20–1.14 (m, 2H); ^{13}C NMR (126 MHz) δ 172.0, 140.9, 131.5, 130.4, 129.4, 129.2, 123.6, 123.5, 61.4, 55.8, 55.7, 55.6, 50.0, 43.9, 39.9, 38.8, 36.6 (3), 35.3, 33.7 (3), 32.5, 28.6, 27.7 (3), 27.4, 26.9, 21.7, 20.3, 19.8; ESI-HRMS: *m/z* Calcd for $C_{33}H_{47}O_3N_3F_3S$ [M+H]$^+$, 622.3284. Found, 622.3278.

N'-Rimantyl-12N-p-trifluoromethlybenzenesulfonyl *matrinicamide hydrochloride* (**7o**): Yield: 58 %; m.p.: 159–160 °C; ^{1}H NMR (500 MHz) δ 10.52 (s, 1H), 8.06 (t, *J* = 8.6 Hz, 2H), 7.96 (dd, *J* = 8.6, 3.6 Hz, 2H), 7.47–7.34 (m, 1H), 4.22–4.04 (m, 3H), 3.82–3.69 (m, 2H), 3.62–3.54 (m, 1H), 3.44 (td, *J* = 9.6, 6.9 Hz, 1H), 3.24–3.16 (m, 2H), 2.96–2.84 (m, 2H), 2.33–2.18 (m, 2H), 2.06–1.94 (m, 2H), 1.92–1.86 (m, 4H), 1.70–1.50 (m, 13H), 1.48–1.36 (m, 7H), 1.25–1.21 (m, 1H), 0.86 (dd, *J* = 8.4, 6.9 Hz, 3H).; ^{13}C NMR (126 MHz) δ 171.0, 145.1, 132.4, 127.7 (2), 126.6 (2), 123.5, 62.9, 57.3, 54.6, 54.5, 51.9, 48.5, 38.4, 38.0 (3), 36.7 (3), 35.5, 35.4, 34.9, 34.5, 27.8(3), 24.8, 24.2, 21.8, 18.3, 18.2, 14.3; ESI-HRMS: *m/z* Calcd for $C_{34}H_{49}O_3N_3F_3S$ [M-HCl+H]$^+$, 636.3441; Found, 636.3443.

N'-Rimantyl-12N-m-trifluoromethlybenzenesulfonyl *matrinicamide hydrochloride* (**7p**): Yield: 51 %; m.p.:146–147 °C ; ^{1}H NMR (500 MHz) δ 10.55 (q, *J* = 9.6 Hz, 1H), 8.19–8.13 (m, 1H), 8.09–8.02 (m, 2H), 7.86 (t, *J* = 7.8 Hz, 1H), 7.46–7.32 (m, 1H), 4.22–4.08 (m, 1H), 3.82–3.73 (m, 2H), 3.64–3.55 (m, 3H), 3.48–3.39 (m, 1H), 3.23–3.16 (m, 2H), 2.90 (tt, *J* = 9.0, 3.4 Hz, 2H), 2.32–2.23 (m, 2H), 1.96 (dd, *J* = 8.6, 6.0 Hz, 1H), 1.92–1.84 (m, 5H), 1.70–1.51 (m, 13H), 1.46–1.36 (m, 6H), 1.32–1.13 (m, 2H), 0.85 (dd, *J* = 10.7, 6.9 Hz, 3H); ^{13}C NMR (126 MHz) δ 170.9, 142.7, 130.9, 130.1, 129.9, 129.6, 123.4, 123.0, 63.1, 57.7, 54.6, 54.5, 51.9, 48.7, 38.4 (3), 38.0, 36.7 (3), 35.5, 35.4, 34.7, 34.2, 27.8 (3), 24.7, 24.4, 21.9, 18.3, 18.2, 14.2; ESI-HRMS: *m/z* Calcd for $C_{34}H_{49}O_3N_3F_3S$ [M-HCl+H]$^+$, 636.3441; Found, 636.3443.

3.2. Biology

3.2.1. Anti–H3N2 effect in vitro

Infuenza A/hanfang/359/95 (wild H3N2) virus and MDCK cells were purchased from ATCC (the American Type Culture Collection). Each compound was dissolved in DMSO at an initial concentration of 20 mg/mL and then diluted threefold successively to obtain 8 different concentrations as stock solutions for the following experiments. MDCK cells were seeded in 96–well trays and cultured at 37 °C in a humidified CO_2 incubator (95% air, 5% CO_2) for 24 h. Then, the cells were infected with influenza H3N2. All infected tissue culture plates (96 wells) were incubated at 37 °C for 2 h, and then the medium was removed. Subsequently, different concentrations of drug maintain solutions were added to the wells (one per well), and the plates were incubated again for 40 h at 37 °C. Then, the inhibition of the virus–induced CPE for each sample was recorded relative to the cell control and the virus control. The IC_{50} values of active compounds were calculated accordingly. The AH was as positive control.

3.2.2. Cytotoxicity assay

MDCK cells were seeded in 96–well trays, each well 25,000 cells and cultured at 37 °C in a humidified CO_2 incubator (95% air, 5% CO_2) for 24 h. Three times dilution compound was added cell monolayer, and continue to cultivate 48 h, then observe the cytopathic record results. The TC_{50} values of active compounds were calculated by the methods of Reed & Muench.

3.2.3. Anti–H3N2 activity assay at protein level

MDCK cells were plated into 6–well culture plates for incubation of 16 h. The medium was removed, and cells were infected with influenza A/hanfang/359/95(H3N2) virus at a MOI of 0.003 for 2 h. Then, various concentrations of the tested compounds were supplemented immediately for incubation of another 24 h. The cells were harvested for western blot assay.

3.2.4. Time-of-addition assay

MDCK cells were infected with infuenza A/hanfang/359/95 (wild H3N2) virus (90 IU/cell) and simultaneously treated with compound or solvent control. After being treated with indicated time (–2 h, 0 h, 2 h, 4 h, and 6, respectively), the cells were washed with PBS and fresh cultural media were added to continuously incubate the cells. Total intracellular proteins were extracted with lysis buffer in 72 h and detected with western blot at 8 h p.i..

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